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ADDITIONAL EVIDENCE OF MUTATION IN
OENOTHERA¹

HARLEY HARRIS BARTLETT

(WITH SEVENTEEN FIGURES)

Introduction

Much of the advance which has been made in genetics and practical breeding during the last decade has been a direct result of the promulgation by DE VRIES of the theory of the origin of species and varieties by mutation. That recessive Mendelian variations originate singly by mutation has been shown by several investigators, notably by MORGAN, who has observed the origin of more than 150 such variations in his cultures of *Drosophila*. Many opponents of the mutation theory deny, however, that progressive mutations ever occur in homozygous strains, or that true species, differing from the parent in several independent characters, have ever been observed to originate at a single step by mutation. DAVIS,² for example, is in accord with the mutationists in regarding *Oenothera gigas* as a marked progressive mutation of specific rank, but he denies that *Oenothera Lamarckiana*, the parent form of *O. gigas*, is homozygous. The facts (1) that *O. Lamarckiana* is not known as a native component of any flora, (2) that its known history has been that of a cultivated plant or an escape from cultivation,

¹ Published by permission of the Secretary of Agriculture.

² DAVIS, B. M., Cytological studies on *Oenothera*. III. A comparison of the reduction divisions of *Oenothera Lamarckiana* and *O. gigas*. Ann. Botany 25:941-974. 1911. "*Oenothera gigas* is a progressive mutant, its peculiarities being clearly associated with the changes in its germ plasm incident upon the doubling of its chromosome number" (*op. cit.* p. 974).

and (3) that its habit of throwing off marked germinal variations is paralleled by the behavior of certain interspecific hybrids in the F_2 and F_3 generations seem to DAVIS³ a sufficient indication that this plant is of comparatively recent hybrid origin, and that its mutations are due to germinal instability resulting from hybridization. He holds that the germinal variations of *O. Lamarckiana* and of various hybrids which he has studied show marked progressive evolution which seemingly cannot be accounted for on a Mendelian basis. Although he does not deny that slight discontinuous variations may occur in homozygous strains (and he insists that the term mutation ought to be used only for such variations), he is of the opinion that variations large enough to be of evolutionary significance occur rarely if at all except in heterozygous lines.

GATES⁴ does not believe that *O. Lamarckiana* is a recent interspecific hybrid, but does ascribe its mutations to germinal instability caused by occasional random crossing with other types. In their main conclusion, that when germinal variation occurs it usually follows crossing, DAVIS and GATES appear to agree. GATES, however, is more emphatic than DAVIS in his conclusion that mutation in *Oenothera* is not merely a result of Mendelian redistribution of unit characters, but is a distinct type of variation. He believes, moreover, that mutation sometimes takes place in pure as well as in hybrid lines. Since the phenomena are identical in the two cases, he has laid especial stress on the fact that there is no excuse for confusing mutation, when it occurs in hybrids, with any type of Mendelian segregation.⁵

³ DAVIS, B. M., Genetical studies on *Oenothera*. II. Amer. Nat. 45:193-233. 1911; III. *Ibid.* 46:377-427. 1912; IV. *Ibid.* 47:449-476, 547-571. 1913.

⁴ GATES, R. R., Mutation in *Oenothera*. Amer. Nat. 45:577-606. 1911.

———, A contribution to a knowledge of the mutating *Oenotheras*. Trans. Linn. Soc. Lond. II. Bot. 8:1-67. 1913.

———, Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. 33:92-99, 113-150. 1913.

⁵ In this author's last paper he says: "The cytological evidence shows that germinal changes may and do occur which are independent of all the laws of hybrid combination and hybrid splitting. This generalization is of more fundamental significance than might at first appear; for it shows that mutation in *Oenothera* is a process *sui generis*, and that no amount of hybrid combination and splitting, Mendelian or otherwise, is sufficient to account for it." GATES, R. R., Breeding experiments which show that hybridization and mutation are independent phenomena. Zeitschr. Ind. Abstammungs- u. Vererbungslehre 11:209-279. 1914.

HERIBERT-NILSSON⁶ has made the first serious effort to explain the variations of *Oenothera Lamarckiana* on a strictly Mendelian basis. He does not hold with DAVIS that this species is necessarily of hybrid origin, but rather that it is a collective species, embracing a number of different strains which constantly cross among themselves. Consequently he assumes that the mutation phenomena do not exemplify progressive and regressive species formation, but merely the synthesis of new combinations from factors already existing within the species.

Although it is by no means true, as some critics seem to imply, that the mutation theory must stand or fall on the evidence derived from *Oenothera*, it must nevertheless be admitted that failure to find a parallel among other more fortunately chosen species of this genus to the mutation phenomena shown by *O. Lamarckiana* would discredit, if not invalidate, much of the direct evidence of mutation which has been so laboriously won by DE VRIES. DAVIS⁷ has said that "it is evident that the adherents of the mutation theory are sensitive to the doubts freely expressed concerning the status of *Oenothera Lamarckiana*, the behavior of which in throwing off marked variants is cited as the most important evidence for the origin of species by mutations. . . . Consequently, mutationists are likely to bring forward as rapidly as possible any evidence that may seem to indicate the appearance of clear inheritable variations of a marked character in forms of pure germinal constitution, i.e., in homozygous material."

It is the object of this paper to present additional evidence of mutation in *Oenothera*, derived from one of the small-flowered, self-pollinating wild American types. Before proceeding farther, however, it should be stated that a considerable body of similar evidence has already been obtained.

DE VRIES⁸ and STOMPS⁹ have twice observed the origin of a dwarf variety of *O. biennis* by mutation, once in a pure line of

⁶ HERIBERT-NILSSON, N., Die Variabilität der *Oenothera Lamarckiana* und das Problem der Mutation. Zeitsch. Ind. Abst. u. Vererb. 8:89-231. 1912.

———, *Oenothera* Problemet. Svensk. Bot. Tidskr. 7¹: pp. 16. 1913.

⁷ DAVIS, B. M., Mutations in *Oenothera biennis* L? Amer. Nat. 47:116-121. 1913.

⁸ DE VRIES, H., Die Mutationen in der Erblchkeitslehre. pp. 28-30. 1912.

———, Gruppenweise Artbildung. pp. 299-306. 1912.

⁹ STOMPS, THEO. J., Mutation bei *Oenothera biennis* L. Biol. Centralbl. 32:521-535. 1912.

O. biennis var. *leptomeris*,¹⁰ and once in a cross between this variety and typical *Oenothera biennis*, from which var. *leptomeris* itself doubtless arose by mutation. STOMPS has also described *O. biennis* mut. *semigigas* from the same culture of *O. biennis* var. *leptomeris* × *O. biennis* which gave rise to the dwarf. A recent letter from Professor DE VRIES (dated May 16, 1914) states that mutations from *O. biennis* are still being obtained at Amsterdam.

STOMPS¹¹ has just published a second report on mutations in *O. biennis*. He records the origin by mutation, in a pure line, of *O. biennis* var. *sulfurea* De V. (long known as a wild component of the Dutch flora), together with mut. *nanella* and mut. *semigigas*. GATES¹² has likewise announced the discovery of mutations (*O. biennis lata*, *O. biennis laevifolia*, *O. biennis rubrinervis*) from *O. biennis*, but has not yet published a full account of his cultures.¹³ Finally, DE VRIES has obtained two different mutations, *O. salicifolia* and *O. salicastrum*, from wild seed of a strain of the self-pollinating *O. biennis* "Chicago" which he collected near Courtney, Missouri; and the writer¹⁴ has given a preliminary account of *Oenothera stenomeris* mut. *lasioptala*,¹⁵ a hairy-petaled derivative of one of the small-flowered cruciate *Onagras*.

¹⁰ *Oenothera biennis* var. *leptomeris* Bartlett. Amer. Jour. Bot. 1:242. 1914 = *Oenothera biennis* var. *cruciata* De Vries, not T. & G.

¹¹ STOMPS, THEO. J., Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179-188. 1914.

¹² GATES, R. R., Parallel mutations in *Oenothera biennis*. Nature 89:659-660. 1912.

¹³ Since the above was written, an account of the cytology of *O. biennis* mut. *lata* has been received. See GATES, R. R., and THOMAS, NESTA, A cytological study of *Oenothera* mut. *lata* and *O. mut. semilata* in relation to mutation. Quar. Jour. Micr. Sci. 59:523-571. 1914.

¹⁴ BARTLETT, H. H., An account of the cruciate-flowered *Oenotheras* of the subgenus *Onagra*. Amer. Jour. Bot. 1:226-243. 1914.

¹⁵ By an unfortunate oversight this name was published in Amer. Jour. Bot. as *O. stenopetala* mut. *lasioptala*. The writer had originally used the name *O. stenopetala* for the species which was described as *O. stenomeris*. After the manuscript had been submitted to the editor, a change was made necessary by the publication of *O. stenopetala* Bicknell, Bull. Torr. Bot. Club 41:79. 1914. In one place the original name escaped notice and was not corrected. It is hoped that the error will not lead to any confusion.

It is shown in this paper (1) that the phenomena of mutation are as characteristic and as easily observed in one of the wild small-flowered self-pollinating *Onagras* as in *Oenothera Lamarckiana*; (2) that the mutations show characters unlike those of any other form with which the parent could have crossed; and (3) that the mutations cannot be ascribed to Mendelian segregation as at present understood. It therefore seems in the highest degree probable that mutation is a phenomenon which is independent of hybridization, and that the evidence of mutation which DE VRIES has found in *Oenothera Lamarckiana* is just as valid as though that species were known as a wild plant and not suspected of having had a horticultural origin.

Differential germination

Several of the most interesting mutations which were observed during the season of 1913 were found quite by chance. One lot of potting soil, in which the seeds of several strains were sown, proved to be a very stiff clay on which a hard crust formed. Germination was so poor that in several cases less than a dozen seedlings resulted from sowing perhaps a thousand or more seeds. It was afterward found that the seeds showed the usual percentage of germination when sown in good soil. In three different species the small progenies obtained when the seeds were planted under unfavorable conditions disclosed striking mutations, which had survived as a result of differential or selective germination. These mutations might easily have been overlooked in a seed pan containing several hundred seedlings, of which only a few were to be retained and grown to maturity.

The three mutant species were from widely separated localities. The seeds of one, from Plymouth, Massachusetts, were sent by Professor B. M. DAVIS; the others were collected by the writer at White Sulphur Springs, W.Va., and Lexington, Ky., respectively. The mutations of the two former species were lost before they matured. It will be useless, therefore, to give an account of their characters or of the cultures in which they appeared until they shall have been found again. In the case of the third species,

O. pratincola, the mutations were brought to maturity and have yielded a second generation. This species, therefore, has been systematically examined for variations, with the results recorded in this paper.

The *Oenothera* population at Lexington, Kentucky

During a brief visit in October 1912, the writer was able to find only two species of *Oenothera* × *Onagra* at Lexington, Ky. They are both new and are referred to below under the names *O. pratincola* and *O. numismatica*. If any other species occur within two or three miles of the city, they must be very scarce. Of course, in October many plants were through blooming and not in such condition that any differences among them would show to the best advantage. Nevertheless, it is believed that no common species could have been overlooked. Nine seed collections were made from individual plants, which showed as great a range of variation as possible. These plants, and the strains descended from them, have been designated by letters from A to I. Eight of the strains proved to be taxonomically identical and are referred to as *O. pratincola*. Lexington A, B, and C were collected in a pasture near Town Creek, 2 miles west of Lexington, where they grew within 200–300 feet of each other. Lexington E, F, G, H, and I were collected at random in vacant lots and within a mile of the city on the west. Lexington D is the only strain of the 9 which is referred to *O. numismatica*. The parent plant grew by a roadside about 2 miles east of Lexington. In addition to the seed collections, many rosettes were collected which flowered in Washington in 1913. Thirteen plants from the same general region as plants E to I proved on flowering to be typical *O. pratincola*, as were also 26 plants from the edge of a field near the reservoir east of the city. It thus appears that *O. pratincola* constitutes the bulk of the *Oenothera* population at Lexington. *O. numismatica* is much scarcer; it did not occur at all among the rosettes which were collected, and was seen in flower only east of Lexington.

The salient characters of the two evening primroses obtained at Lexington are the following:

In *O. pratincola*

A well-grown plant, is 1.5 m. high, and loosely branched.

The basal branches are frequently simple.

The flowering time lasts about six weeks.

The lax terminal spike often becomes 5-6 dm. long (see fig. 1).

The lateral branches below the terminal spike are few in number and become 4-5 dm. long.

The lowest bracts of the upper lateral spikes are ovate, and grade upward to lanceolate.

The calyx segments are so sparsely pilose as to appear practically glabrous.

The hairs of the calyx segments are about 1 mm. long, thick-walled, acute, with multicellular tuberculate bases.

In *O. numismatica*

A well-grown plant, is about 1 m. high, and densely branched.

The basal branches bear tertiary branches and resemble the main stem.

The flowering time lasts only about two weeks.

The dense terminal spike is about 2 dm. long in fruit (see fig. 2).

The lateral branches below the terminal spike are numerous and are seldom over 2 dm. long.

The lowest bracts of the upper lateral spikes are nearly orbicular and grade upward through oblong to lanceolate (see fig. 2).

The calyx segments are closely and finely pubescent.

The hairs of the calyx segments are less than 0.5 mm. long, and belong to two types: (1) an acute thick-walled type without tuberculate bases, and (2) a thin-walled, round-ended, clavate or cylindrical type.

Technical diagnoses of these two species, together with a discussion of their possible relationships, have been published elsewhere.¹⁶ *O. pratincola* appears to be a frequent plant in the North Central States. *O. numismatica*, on the contrary, is known only from Lexington and may well be a local species, possibly derived by mutation from *O. pratincola*. Its close resemblance in certain characters to one of the mutations of *O. pratincola* is pointed out elsewhere in this paper.

The mutations of "Lexington C"

Seeds from four of the parent plants of *O. pratincola* which had been selected at Lexington were planted early in the spring of 1913.

¹⁶ BARTLETT, H. H., Twelve elementary species of *Onagra*. *Cybele Columbiana* 1:37-56. 1914.

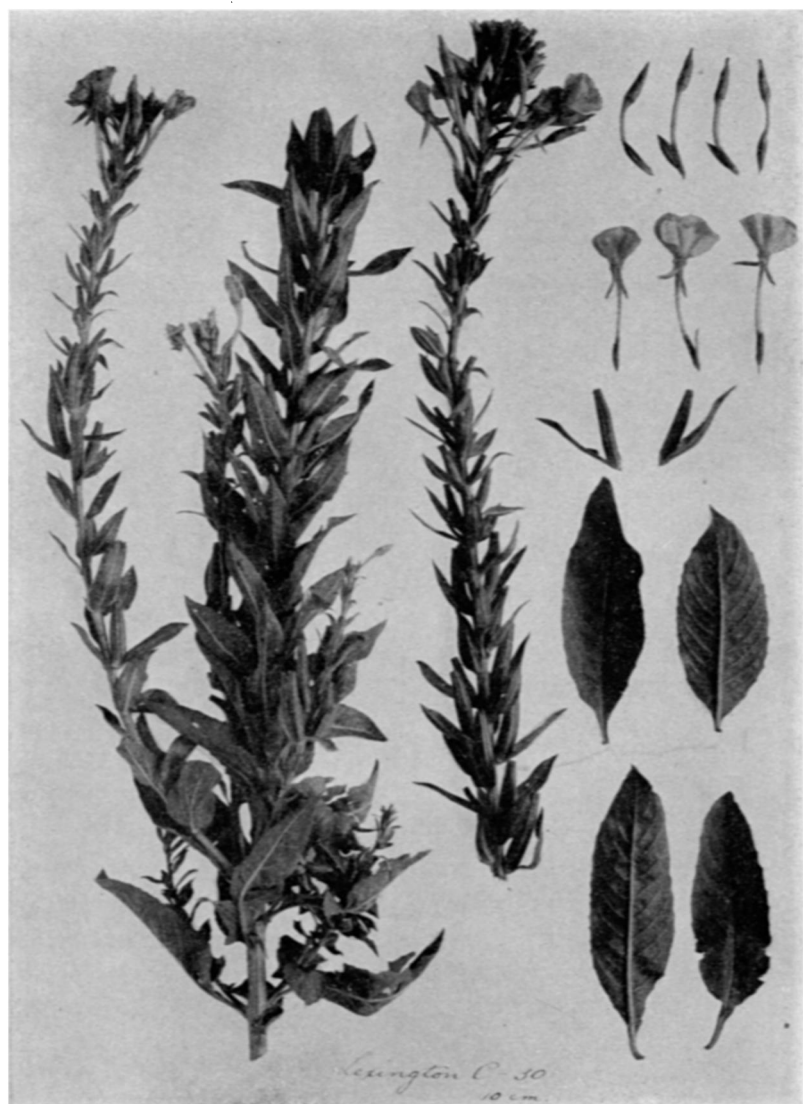


FIG. 1.—*Oenothera pratincola*, sp. nov.: upper part of main stem of Lexington C-30; leaves from middle of stem; flowers and buds; $\times \frac{1}{2}$.



FIG. 2.—*Oenothera numismatica*, sp. nov.: upper part of main stem of Lexington D-29; leaves from middle of stem; lateral branches from just below the terminal spike; characteristic foliage of such a lateral branch; in contrast with fig. 1, note the denser shorter spikes, which are only in flower a short time, the much closer branching, and the characteristic suborbicular leaves of the uppermost lateral branches; $\times \frac{1}{2}$.

Lexington A, B, and E germinated readily. Since no variation was noticed among the hundreds of seedlings of these three strains, all were discarded except 30 of each, which were potted off for the field cultures. The seeds of Lexington C, however, had been planted too deeply in unsuitable clay soil, and, although the seed

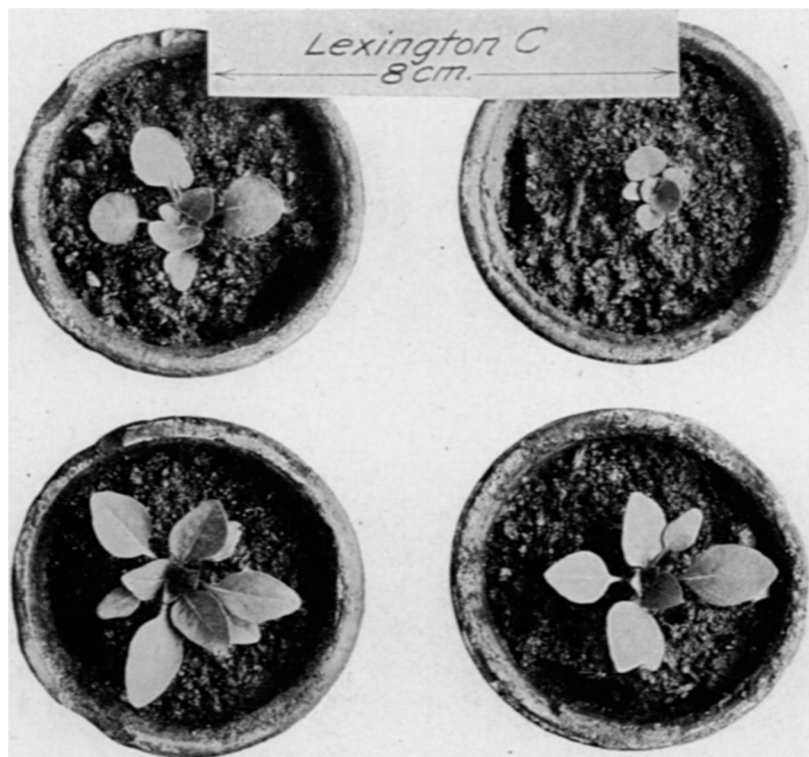


FIG. 3.— F_1 progeny of *Oenothera pratincola*: four of the 9 seedlings which constituted the first culture of Lexington C; the 2 upper plants, Lexington C-1 and C-2, are mut. *nummularia*; the latter bore seeds which gave rise to the F_1 culture referred to in table I; the 2 lower plants are typical *O. pratincola*.

pan received the same treatment as the rest, weeks passed before any seedlings appeared. At length 9 plants were obtained which were potted off. Almost from the first, they showed remarkable variation among themselves. Six (nos. 3, 4, 5, 6, 8, and 9) were typical *O. pratincola*, and agreed in all characters with the seed-

lings of Lexington A, B, and E; one (no. 7) was of a darker green color than the type, the leaves were somewhat narrower, and the petiole and midrib below the middle of the blade were particularly broad and white; two (nos. 1 and 2) had almost orbicular leaves, and constituted the most striking deviation from the expected form

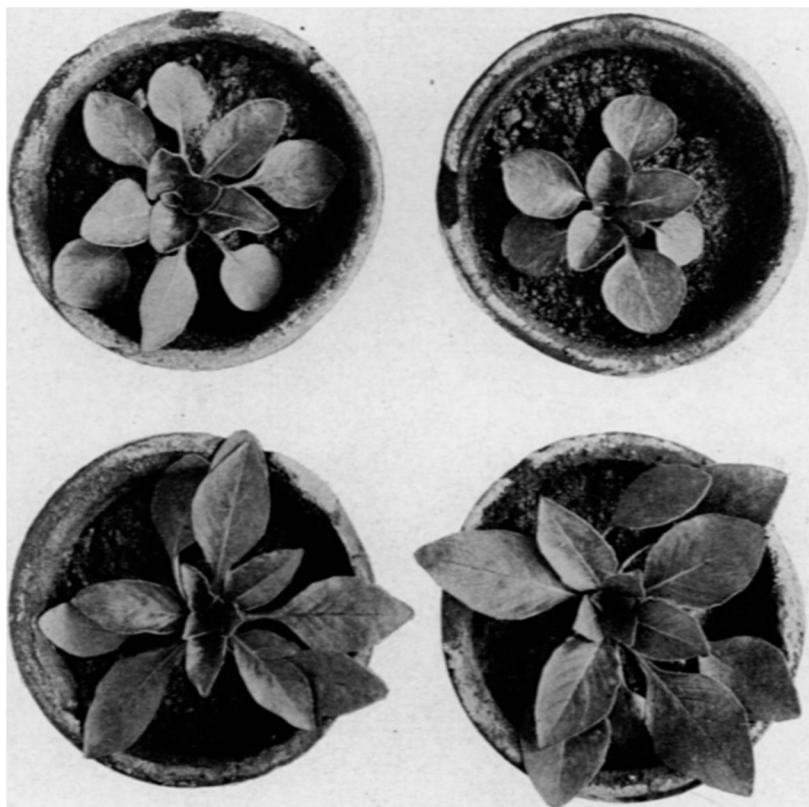


FIG. 4.— F_1 progeny of *Oenothera pratincola*, Lexington C: the same plants shown in fig. 3, but a month older.

that the writer had ever observed in a seedling of *Oenothera*. In fig. 3 the two upper plants are the round-leaved variations, nos. 1 and 2; the two lower are typical *O. pratincola*, nos. 3 and 4. In fig. 4 the same 4 plants are shown when a month older. The reader will observe that the orbicular seedling leaves of nos. 1 and 2 have

been superseded in the more mature rosette by leaves of a different form, but that the sharp distinction between the variation and the typical form has not been obscured. The occurrence of so interesting a variation in Lexington C led to a careful examination of the strain for evidence of mutability.

A second sowing of the same seed on good soil resulted in a progeny of 720 seedlings from 1000 seeds. As soon as the seedlings were well rooted, they were transplanted to square seed pans in which they were widely enough spaced to allow of unimpeded growth for a month or six weeks. This system was followed in all subsequent work. Of course, the seeds were invariably sown on sterilized soil. After the seedlings were transplanted, the pans were frequently examined for mutations, and all plants which were noticeably divergent from the mass of the culture were marked for preservation. Among the 720 seedlings of the second sowing, there were only 4 round-leaved plants. Since the mass of the culture was uniform, and the round-leaved plants constituted an absolutely discontinuous variation from both the typical form and one other pronounced variant which occurred in the culture, it was concluded that they were probably mutations. In the following pages the round-leaved type is called *O. pratincola* mut. *nummularia*.¹⁷

In order to show the discontinuity between typical *O. pratincola* and mut. *nummularia*, photographs of two of the seed pans in which this mutation occurred are reproduced as figs. 5 and 6. At the time the pans were photographed, the plants were about as far advanced as nos. 1-4 in fig. 3. Comparison of the figures will

¹⁷ The writer has suggested (Amer. Jour. Bot. 1:237. 1914) that mutations of experimental origin be given trinomial names such as *O. pratincola* mut. *nummularia*, in order to avoid confusion with names which must be given consideration in floristic works. A trinomial nomenclature has the advantage over the binomial system proposed by GATES (Trans. Linn. Soc. London II. Bot. 8:10. 1913) in that the parallelism of mutations occurring in different species may be indicated by the use of the same mutational designation. For example, a convenient way to show the parallelism between the mutations of *O. Lamarckiana* and those of *O. biennis* would be to call them *O. Lamarckiana* mut. *semigas*, *O. biennis* mut. *semigas*, etc. The trinomial used in this way need imply nothing as to the specific, varietal, or formal rank of a mutation, but only the manner of its origin. Nevertheless, for the sake of avoiding confusion, it would be well not to give any mutation a name which had previously been used in any subspecific category within the species which had given rise to the mutation.

show the complete identity, at this stage of growth, of different individuals of mut. *nummularia*, and also the great uniformity of the typical plants of the culture.



FIG. 5.— F_2 progeny of *Oenothera pratincola*, Lexington C, pan 1: the only mutation shown is Lexington C-10, mut. *nummularia*; the other plants are typical *O. pratincola*; about the same age as the plants shown in fig. 3.

In addition to the 4 plants of mut. *nummularia* which were discovered in the second sowing, there were solitary specimens of each of two other mutations, one plant (no. 12) like no. 7 of the first planting, and another (no. 18) unlike anything else in the culture. No. 18 had exceedingly narrow, red, subulate seedling leaves and

was called on this account mut. *subulata*. There were also 7 plants (nos. 13-16, 19, 20, and 22) which developed very slowly and were retained in the expectation that they might prove to be dwarfs, although there was no character but size to distinguish



FIG. 6.—F₁ progeny of *Oenothera pratincola*, Lexington C, pan 2; the only mutation shown is Lexington C-II, mut. *nummularia*; the other plants are typical.

them from typical sister plants. The solitary plant of mut. *subulata* died, but the type has since been found to be one of the most frequent mutations of *O. pratincola*. The type represented by nos. 7 and 12 was designated as mut. *pusilla*. Its rosettes were about 4 cm. in diameter at maturity. The stem leaves were

linear-lanceolate. The stems were simple. No. 7 died just before flowering, when only 7 cm. high. No. 12 flowered at a height of 10 cm. The ovary was 7 mm. long; hypanthium 10 mm. long; calyx segments 4 mm. long, excluding the distant free tips, which were 1 mm. long. The calyx differed from that of the typical form not only in having distant calyx tips, but also in being densely soft-pubescent. Unfortunately, this plant was sterile and produced no seeds. As far as the writer is aware, mut. *pusilla* represents the extreme of nanism in the subgenus *Onagra*.

With a single exception, the 7 suspected dwarfs developed as quite normal plants, indistinguishable from the mass of the culture. One plant, no. 19, differed from the rest in that it had stiff, distant calyx tips 5 mm. long which were continued on the angles of the squarish bud as a marked carina. The buds were almost glabrous, as in the type form of the culture, but in marked contrast to some of the other mutations. This plant was self-sterile, but produced abundant seeds when pollinated with typical *O. pratincola*.

The Lexington C culture which was grown to maturity in 1913 included, besides the 9 plants from the first sowing and the mutations and suspected mutations of the second sowing, all the plants from two pans in which there appeared to be no variation. There were 72 of these plants, nos. 23-94.¹⁸ When they matured two mutations were found which had not been detected in the early seedling stages. With these two exceptions, the plants were absolutely uniform among themselves, and exactly the same as Lexington A, Lexington B, and Lexington E. (Of each of these three strains 30 plants were grown to maturity.) The two mutations were not alike and were different from any of the other new types which had been obtained. Both, however, were almost

¹⁸ The culture numbers of these plants are all given here in order to avoid lengthy repetition in subsequent papers which will deal with the same strains. It may be well to explain that every plant in the writer's garden is designated by the name of the strain (for which a number has often been substituted) followed by a succession of numbers which indicate the pedigree and number in the culture of each individual. Subscripts are used when it is wished to distinguish between sister plants grown in different years, or to indicate the years in which the successive generations were grown. "Lexington C-11₁₃," for example, would be the complete designation of the plant of mut. *nummularia* which is shown in fig. 6. Plants of the F₂ generation, grown in 1914, would be "Lexington C-11₁₃-14," "Lexington C-11-2," etc.

self-sterile. No. 28 was half as high as typical plants of the culture; the leaves were broader and white-margined; the buds were smaller and closely viscid-puberulent with a hair type which does not occur in the typical form; the branching differed in that there



FIG. 7.—A, *Oenothera pratincola* mut. *nummularia*, Lexington C-21; B, *Oenothera pratincola* (typical), Lexington C-16; the 2 plants are of the same age and were grown under identical conditions; note particularly the difference in the branching.

were numerous inflorescence-bearing tertiary branches. No. 57 (mut. *nitida*) was slightly taller than no. 28, almost simple, with leaves narrower than in the typical form, upwardly rolled parallel to the mid-vein, very glistening, nearly twice as thick as in normal

plants, and very brittle. A few seeds were obtained from this plant by self-pollination, but they did not germinate.

The 729 plants of Lexington C which were grown in 1913 yielded in all 6 different mutations. All of them except mut. *nitida* and mut. *nummularia* were infertile or nearly so to their own pollen. The latter type, in spite of the fact that it was unwittingly subjected to very unfavorable conditions, produced seeds from which a second generation has been grown. For fear of losing the 6 original plants, they were planted in the center bed of the greenhouse when the rest of the culture was transferred to the garden. Before it was realized how much their development was being retarded by the extreme heat in the greenhouse, it was too late to move them again with any prospect of success. Three plants died after they had begun to flower, but before any seeds were ripe.

The characters of mut. *nummularia*

A few plants of typical *O. pratincola* which were kept in the greenhouse with the mutation served to show that there are distinct differences in the habits of growth of the two types, when they are grown under identical conditions. This fact will be apparent from fig. 7, in which two sister plants of the same age are shown. It will be noticed that the stature of the mutation is less than that of the parent type, but that the lateral branches are more numerous and more densely leafy. A thoroughgoing comparison of the two types cannot be made until the cultures of 1914 shall have grown to maturity out of doors. The more striking contrasting characters, however, are the following:

In *O. pratincola*

The early seedling leaves are ovate.

The stem leaves are reflexed.

The lower leaves of the lateral branches are ovate-lanceolate.

The ovary and calyx are sparsely pilose (sometimes almost glabrous).

In mut. *nummularia*

The early seedling leaves are orbicular.

The stem leaves are involute.

The lower leaves of the lateral branches are broadly ovate.

The ovary and calyx are closely and finely pubescent.

In *O. pratincola*

The hairs of the calyx segments and hypanthium are about 1 mm. long and all belong to the thick-walled, acute type with multicellular, tuberculate bases.

The calyx segments separate in pairs.

In mut. *nummularia*

The hairs of the calyx segments and hypanthium are less than 0.5 mm. long and belong to two types: (1) acute, thick-walled hairs without multicellular, tuberculate bases, and (2) thin-walled, round-pointed clavate or cylindrical hairs.

The four calyx segments remain united when the flower opens.

The difference in the rupture of the calyx is shown in fig. 8. The writer is inclined to believe that the clear-cut qualitative distinction between the calyx pubescence of the parent form and that of the mutation will provide an absolute criterion for determining whether or not mut. *nummularia* marks an evolutionary advance over *O. pratincola*. A priori it seems to be a safe prediction that mut. *nummularia* will prove to be a progressive mutation of even more striking individuality than *O. gigas*.

It is unfortunate that data on reciprocal crosses between *O. pratincola* and mut. *nummularia* will not be available until next year. The first flowers of the original mutations were, of course, self-pollinated, and further work was prevented by the loss of the plants. This year (1914) the writer has numerous plants of mut. *nummularia* (primary mutations as well as F_1 plants) with which to make the necessary crosses.

The heritability of mut. *nummularia*

The three individuals of mut. *nummularia* which bore seeds were nos. 2, 17, and 21. Even these, however, wilted and dried up while still in flower, so that very few capsules were obtained. As in the case of many somewhat self-sterile *Oenotheras*, the capsules were small and contained few good seeds. From each of several capsules only one or two seeds were obtained, and the best had but 30, whereas a large capsule of typical *O. pratincola* contains well over 300. Until plants of the mutation shall have developed under more favorable conditions than those to which the first season's plants were subjected, it will be impossible to say

whether or not mut. *nummularia* is really as nearly self-sterile as this comparison would indicate. At any rate, only 403 seeds, many of them obviously too unripe to germinate, were obtained from 3 plants of the mutation. The seeds have given an F_1 progeny of 135 plants which is now (April 1914) in the early seedling stage.

The F_1 generation from mut. *nummularia* consists in part of plants which exactly reproduce the parental type and in part of secondary mutations. At the time this article is being written the plants are still young, but it is nevertheless clear (1) that the

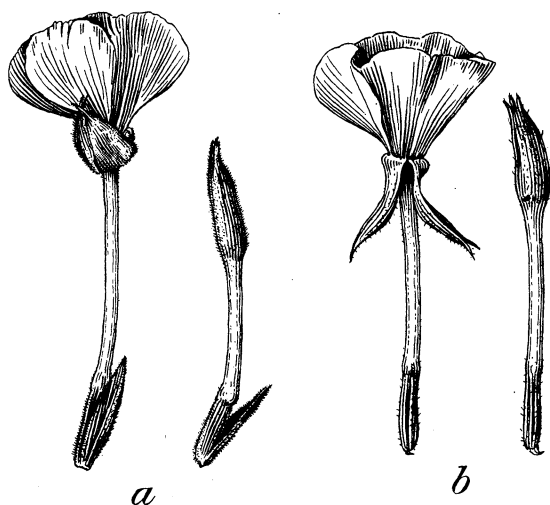


FIG. 8.—Flowers and buds of (a) *Oenothera pratincola* mut. *nummularia*, and (b) typical *O. pratincola*, showing especially the difference in the rupture of the calyx.

F_1 generation includes no typical *O. pratincola*; (2) that the secondary mutations (with one possible exception) are narrower leaved than *O. pratincola* and therefore even more sharply distinguished from mut. *nummularia* than the latter is from *O. pratincola*; and (3) that all of the secondary mutations (again with the single exception noted above) appear to be quite identical with certain primary mutations which have appeared simultaneously with mut. *nummularia* in various cultures of typical *O. pratincola*.

The secondary mutations fall into three well marked groups which have been called mut. *tortuosa*, mut. *rubricentra*, and mut.

subulata. It is of course impossible to establish absolute identities among seedling plants of types which have not yet been seen in flower. Consequently the F_1 progeny of mut. *nummularia* are classified either as true to type or as secondary mutations in table I, which shows the composition of the cultures now under observation.

TABLE I

COMPOSITION OF F_1 GENERATION OF MUT. *nummularia* (SEEDLING STAGE)

| Parent | Number of seeds | Total plants | Mut. <i>nummularia</i> | Secondary mutations |
|----------------------------|-----------------|--------------|------------------------|---------------------|
| Lex. C-2 | 15* | 8 | 6 | 2 |
| “ | 15* | 6 | 5 | 1 |
| “ | 30* | 15 | 15 | 0 |
| “ | 65 | 9 | 7 | 2 |
| Lex. C-2 Total | 125 | 38 | 33 | 5 |
| Lex. C-17 | 39 | 14 | 12 | 2 |
| Lex. C-17 × C-21 | 20* | 10 | 8 | 2 |
| “ | 24* | 2 | 2 | 0 |
| “ | 11* | 5 | 3 | 2 |
| “ | 18* | 5 | 5 | 0 |
| “ | 64 | 25 | 15 | 10 |
| Lex. C-17 × C-21 Total ... | 137 | 47 | 33 | 14 |
| Lex. C-21 | 102 | 36 | 31 | 5 |
| Grand total | 403 | 135 | 109 | 26 |

* Indicates that the seeds were from one capsule.

Table I shows that only 34 per cent of the seeds of mut. *nummularia* germinated. In order to obtain as many plants as possible, a large number of seeds were counted into the seed pans which seemed too immature to germinate; 65 such seeds, planted by themselves, produced 9 plants. Part of the seeds planted were obtained from self-pollinated capsules, others from capsules which had been cross-pollinated. Table I shows that the progeny from the self-pollinated seeds includes secondary mutations and typical *nummularia* plants in the ratio 1:6. The same ratio for the progeny from cross-pollinated seeds is about 1:3.2. Although the difference in the ratio seems very marked, it may be due to the fact that the germination was poor and the cultures small.

Some of the progeny of two of the parents from which F_1 plants were obtained (Lexington C-17 and Lexington C-21) are shown in figs. 9-11. In fig. 9, no. 3 is a young specimen of mut. *tortuosa*, as yet only vaguely suggesting the characters which give this mutation its name. The other 5 plants are typical mut. *nummularia*, comparable in state of development with nos. 1 and 2 in fig. 3, and nos. 10 and 11 in figs. 5 and 6. Fig. 10 shows three of the types which are included in the F_1 cultures from mut. *nummularia*. Nos. 8 and 15 are characteristic plants of mut. *tortuosa*; nos. 13 and 16 are mut. *rubricentra*; nos. 35 and 36 are typical mut. *nummularia*. In order to show the striking uniformity of the *nummularia* plants 6 more of them are shown in fig. 11.

The frequency of mut. *nummularia*

In order to determine the frequency with which *O. pratincola* gives rise to mut. *nummularia*, large cultures were grown in the greenhouse during the winter of 1913-14. As usual, the seeds were sown on sterilized soil and transplanted to seed pans as soon after germination as circumstances permitted.¹⁹ Remaining wild seeds of the original collections gave additional F_1 cultures of Lexington C, A, B, and E. F_1 cultures were also grown from the wild seeds of Lexington F, G, H, and I, which had not been previously planted. It will be remembered that mutations had been detected during the first year of cultivation only in Lexington C, and in this strain only because of the accidental application of the method of selective germination. The other strains were found to be quite as mutable as Lexington C when all of the seedlings were retained until old enough to show their distinctive characteristics. In addition to the F_1 cultures, F_2 cultures were grown from seeds of 8 self-pollinated F_1 sister plants of Lexington C, 1 self-pollinated plant of Lexington A, and 2 self-pollinated plants of Lexington B. These F_2 progenies from guarded seeds were found to contain approximately the same proportion of mutations as the F_1 progenies from unguarded wild seeds.

¹⁹ The writer wishes to express here his appreciation of Mr. MARTIN BILON'S painstaking and efficient care of the germination pans and the young seedlings.

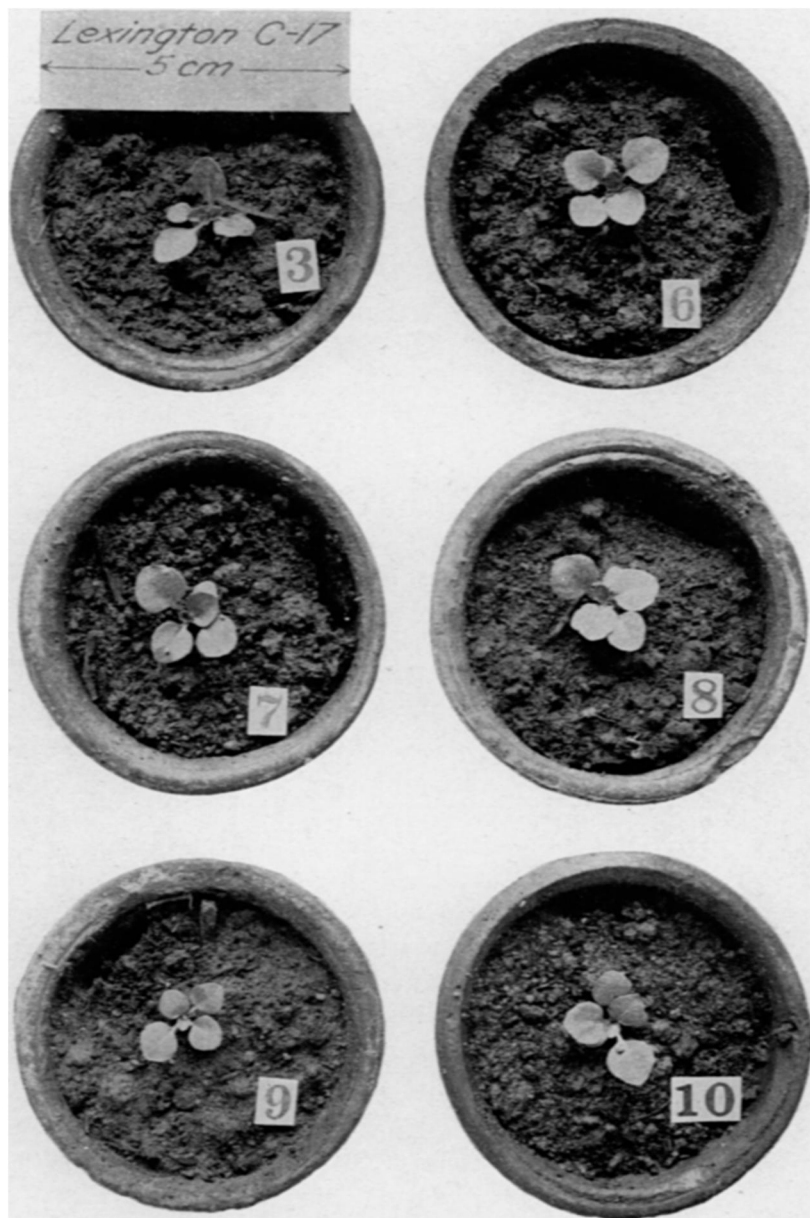


FIG. 9.— F_1 progeny of *Oenothera pratincola* mut. *nummularia*, Lexington C-17; the plant in the upper left-hand corner, Lexington C-17-3, is *O. pratincola* mut. *tortuosa*, here occurring as a secondary mutation, but seemingly the same as one of the very rarest primary mutations of *O. pratincola*; the other plants are typical examples of mut. *nummularia*.

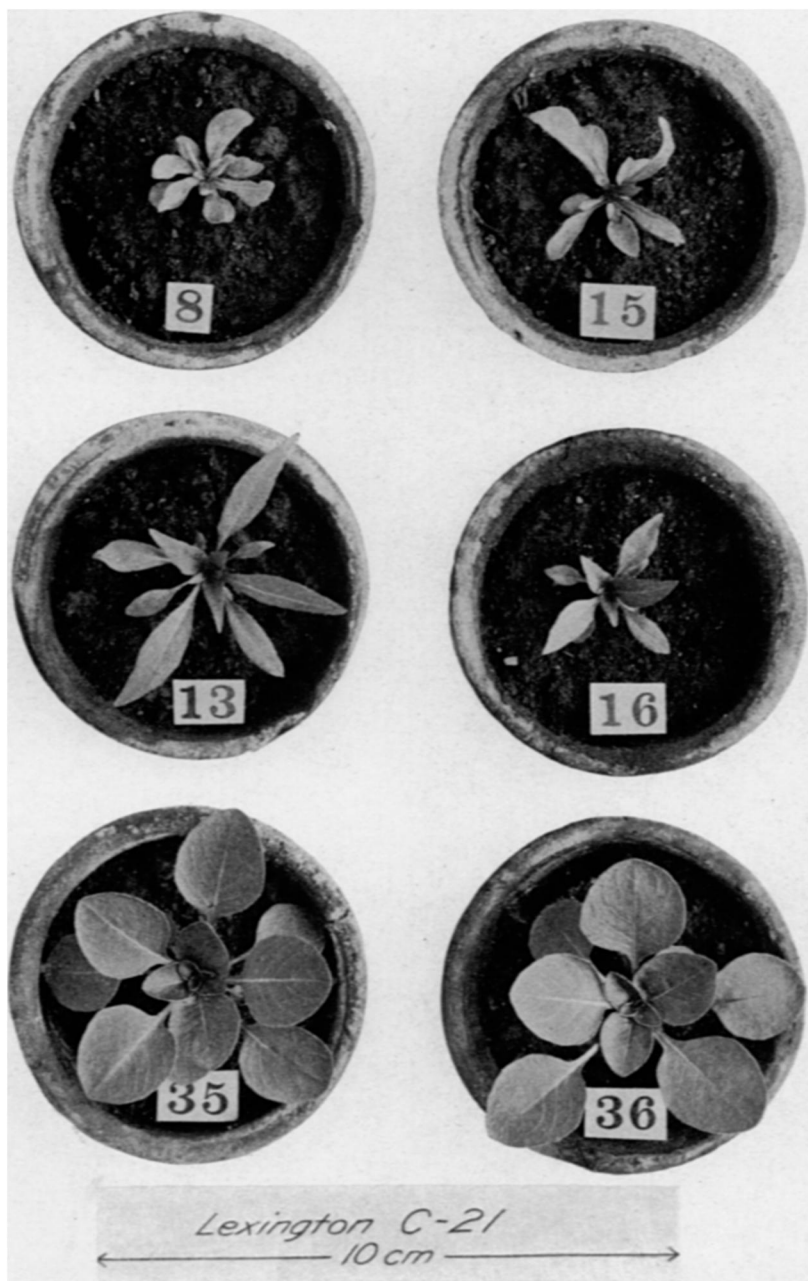


FIG. 10.—F₁ progeny of *Oenothera pratincola* mut. *nummularia*, Lexington C-21; the 2 upper plants, Lexington C-21-8 and C-21-15, are mut. *tortuosa*; the 2 in the middle row, C-21-13 and C-21-16, are mut. *rubricentra*; the 2 below are mut. *nummularia*; the plants shown in this cut are three weeks older than those shown in fig. 9.

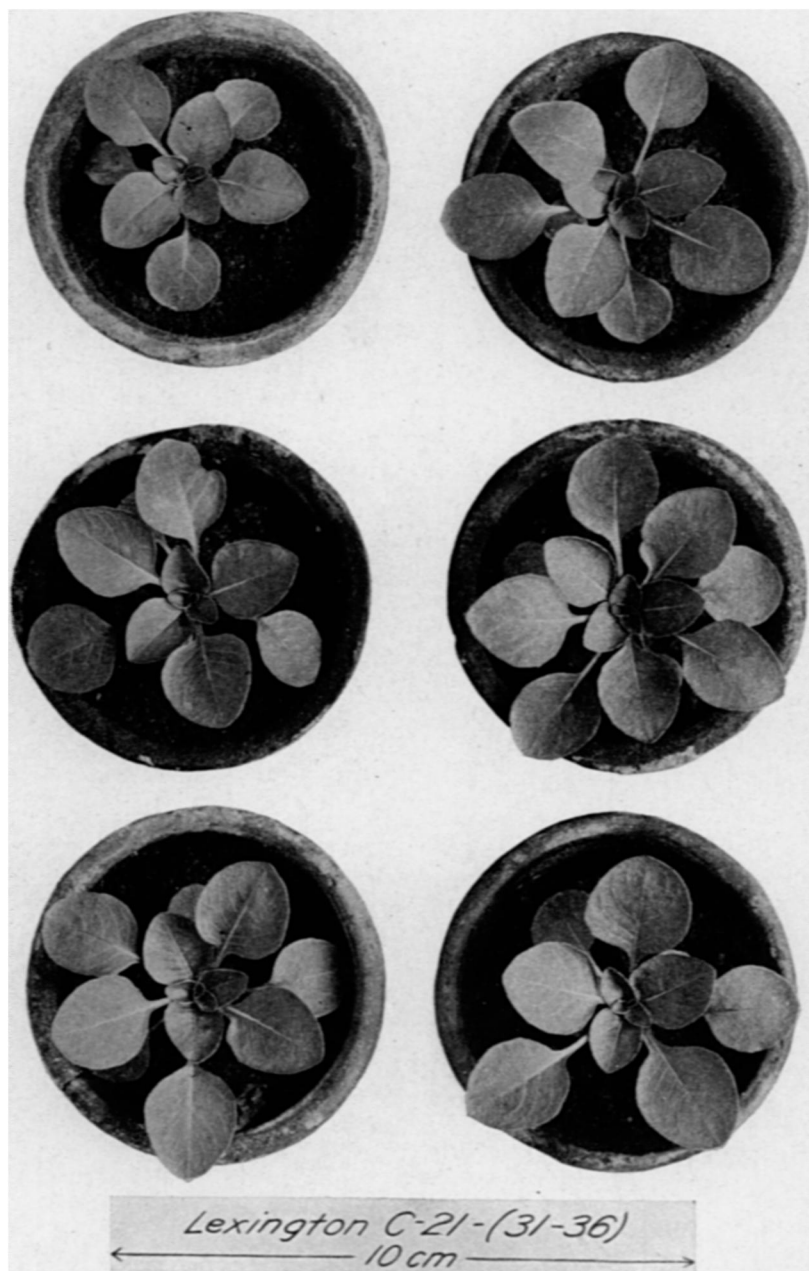


FIG. 11.— F_1 progeny of *Oenothera pratensis* mut. *nummularia*; typical examples of mut. *nummularia*, sister plants of those shown in fig. 10, of the same age.

Data in regard to the frequency of mut. *nummularia* in all the strains of *O. pratincola* except Lexington E are given in tables II-XI, and are summarized in table XII. Lexington E yielded striking mutations in both F_1 and F_2 generations, but they constituted an entirely different series of forms from those which were obtained from the other strains. In several respects the mutation phenomena presented by Lexington E were unique. It will be necessary, therefore, to defer an account of this strain until next year.

It will be noticed from the tables that a large number of seeds were planted capsule by capsule. The variation in number of seeds per capsule appears greater than it should, for in many cases the capsules had dehisced and lost part of their contents. In general, a capsule of *O. pratincola* contains 200-300 seeds.

TABLE II
ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON C"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-------------|---------------|--------------|---------|-----------------------------|---------------------------------|
| 2 | 1000 | 720 | 711 | 4 (nos. 10, 11, 17, and 21) | 5 |
| 3 | 133* | 101 | 93 | 0 | 8 |
| 4 | 130* | 117 | 116 | 0 | 1 |
| 5 | 116* | 111 | 106 | 0 | 5 |
| 6 | 88* | 21 | 20 | 0 | 1 |
| 7 | 125* | 20 | 20 | 0 | 0 |
| 8 | 162* | 152 | 149 | 0 | 3 |
| 9 | 164* | 143 | 140 | 0 | 3 |
| 10 | 237* | 172 | 165 | 0 | 7 |
| 11 | 237* | 223 | 211 | 1 (no. 123) | 11 |
| 12 | 65* | 35 | 35 | 0 | 0 |
| 13 | 217* | 147 | 143 | 1 (no. 136) | 3 |
| 14 | 200 | 156 | 153 | 0 | 3 |
| 15 | 200 | 154 | 152 | 0 | 2 |
| 16 | 147* | 96 | 93 | 0 | 3 |
| 17 | 200 | 60 | 58 | 0 | 2 |
| 18 | 200 | 155 | 152 | 2 (nos. 150 and 151) | 1 |
| 19 | 200 | 98 | 95 | 0 | 3 |
| 20 | 200 | 130 | 124 | 2 (nos. 156 and 160) | 4 |
| 21 | 200 | 112 | 109 | 1 (no. 162) | 2 |
| Total | 4,221 | 2,923 | 2,845 | 11 | 67 |

* Indicates seeds from the same capsule.

TABLE III
ANALYSIS OF F₂ SEEDLING CULTURES OF "LEXINGTON C"

| Parent | Culture | Number of seeds | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-----------------|---------|-----------------|--------------|---------|------------------------|---------------------------------|
| Lex. C-5 | 1 | 244* | 151 | 150 | 0 | 1 |
| " | 2 | 208* | 155 | 154 | 0 | 1 |
| " | 3 | 190* | 140 | 138 | 1 (no. 3) | 1 |
| Lex. C-5 | Total | 642 | 446 | 442 | 1 | 3 |
| Lex. C-6 | 1 | 116* | 41 | 39 | 0 | 2 |
| " | 2 | 106* | 41 | 40 | 0 | 1 |
| " | 3 | 58* | 31 | 27 | 0 | 4 |
| " | 4 | 41* | 35 | 35 | 0 | 0 |
| " | 5 | 21* | 10 | 10 | 0 | 0 |
| " | 6 | 14* | 13 | 13 | 0 | 0 |
| Lex. C-6 | Total | 356 | 171 | 164 | 0 | 7 |
| Lex. C-15 | 1 | 196* | 116 | 115 | 1 (no. 1) | 0 |
| " | 2 | 233* | 115 | 115 | 0 | 0 |
| " | 3 | 173* | 106 | 106 | 0 | 0 |
| " | 4 | 134* | 123 | 122 | 1 (no. 2) | 0 |
| " | 5 | 28* | 12 | 12 | 0 | 0 |
| " | 6 | 31* | 12 | 12 | 0 | 0 |
| " | 7 | 48* | 41 | 41 | 0 | 0 |
| " | 8 | 43* | 32 | 31 | 1 (no. 11) | 0 |
| " | 9 | 12* | 9 | 9 | 0 | 0 |
| " | 10 | 17* | 15 | 15 | 0 | 0 |
| " | 11 | 31* | 15 | 15 | 0 | 0 |
| Lex. C-15 | Total | 946 | 596 | 593 | 3 | 0 |
| Lex. C-22 | 1 | 148* | 122 | 119 | 0 | 3 |
| " | 2 | 203* | 169 | 157 | 1 (no. 9) | 11 |
| " | 3 | 172* | 147 | 142 | 1 (no. 20) | 4 |
| " | 4 | 225* | 189 | 184 | 1 (no. 21) | 4 |
| " | 5 | 250* | 196 | 191 | 0 | 5 |
| Lex. C-22 | Total | 998 | 823 | 793 | 3 | 27 |
| Lex. C-36 | 1 | 182* | 142 | 141 | 0 | 1 |
| " | 2 | 208* | 150 | 147 | 0 | 3 |
| " | 3 | 210* | 159 | 157 | 0 | 2 |
| " | 4 | 231 | 181 | 179 | 0 | 2 |
| " | 5 | 147* | 99 | 96 | 0 | 3 |
| Lex. C-36 | Total | 978 | 731 | 720 | 0 | 11 |
| Lex. C-52 | 1 | 215* | 148 | 146 | 1 (no. 1) | 1 |
| " | 2 | 118* | 100 | 100 | 0 | 0 |
| " | 2 | 189* | 138 | 136 | 0 | 2 |
| " | 4 | 150* | 75 | 73 | 1 (no. 28) | 1 |
| " | 5 | 225* | 173 | 173 | 0 | 0 |
| Lex. C-52 | Total | 897 | 634 | 628 | 2 | 4 |

TABLE III—Continued

| Parent | Culture | Number of seeds | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-----------------------------|-------------|-----------------|--------------|---------|-----------------------------|---------------------------------|
| Lex. C-72 | 1 | 192* | 171 | 165 | 1 (no. 6) | 5 |
| " | 2 | 223* | 180 | 179 | 0 | 1 |
| " | 3 | 235* | 187 | 186 | 0 | 1 |
| " | 4 | 279* | 242 | 241 | 0 | 1 |
| " | 5 | 280* | 230 | 225 | 0 | 5 |
| Lex. C-72 | Total | 1,209 | 1,010 | 996 | 1 | 13 |
| Lex. C-91 | 1 | 185* | 146 | 144 | 2 (nos. 1 and 2) | 0 |
| " | 2 | 284* | 245 | 243 | 1 (no. 4) | 1 |
| " | 3 | 245* | 172 | 171 | 0 | 1 |
| " | 4 | 267* | 125 | 121 | 0 | 4 |
| " | 5 | 316* | 198 | 195 | 0 | 3 |
| " | 6 | 337* | 300 | 293 | 5 (nos. 39, 40, 42, 43, 44) | 2 |
| " | 7 | 242* | 187 | 181 | 0 | 6 |
| Lex. C-91 | Total | 1,876 | 1,373 | 1,348 | 8 | 17 |
| Eight F ₁ plants | Grand total | 7,902 | 5,784 | 5,684 | 18 | 82 |

* Indicates seeds from the same capsule.

TABLE IV

ANALYSIS OF F₁ SEEDLING CULTURES OF "LEXINGTON A"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-------------|---------------|--------------|---------|------------------------|---------------------------------|
| 2 | 341* | 66 | 59 | 0 | 7 |
| 3 | 405* | 129 | 118 | 2 (nos. 44 and 45) | 9 |
| 4 | 337* | 160 | 53 | 2 (nos. 53 and 54) | 5 |
| Total | 1,083 | 255 | 230 | 4 | 21 |

* Indicates seeds from the same capsule.

TABLE V

ANALYSIS OF F₂ SEEDLING CULTURES OF "LEXINGTON A"

| Parent | Culture | Number of seeds | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|----------------|---------|-----------------|--------------|---------|------------------------|---------------------------------|
| Lex. A-5 | 1 | 119* | 76 | 73 | 0 | 3 |
| " | 2 | 200 | 115 | 110 | 0 | 5 |
| " | 3 | 200 | 118 | 115 | 2 (nos. 9 and 11) | 1 |
| " | 4 | 200 | 127 | 122 | 1 (no. 15) | 4 |
| Lex. A-5 | Total | 719 | 336 | 420 | 3 | 13 |

* Indicates seeds from the same capsule.

TABLE VI
ANALYSIS OF F₁ SEEDLING CULTURES OF "LEXINGTON B"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-------------|---------------|--------------|---------|------------------------|---------------------------------|
| 2 | 149* | 107 | 95 | 0 | 12 |
| 3 | 324* | 188 | 175 | 0 | 13 |
| 4 | 247* | 107 | 97 | 0 | 10 |
| 5 | 200* | 106 | 101 | 0 | 5 |
| Total | 920 | 508 | 468 | 0 | 40† |

* Indicates seeds from the same capsule.

† Of the 40 mutations and suspected mutations, 36 were merely smaller plants than the average, selected in the expectation that some might prove to be dwarfs.

TABLE VII
ANALYSIS OF F₂ SEEDLING CULTURES OF "LEXINGTON B"

| Parent | Culture | Number of seeds | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|---------------------------|-------------|-----------------|--------------|---------|------------------------|---------------------------------|
| Lex. B-1 | 1 | 184* | 157 | 157 | 0 | 0 |
| " | 2 | 269* | 142 | 137 | 0 | 5 |
| " | 3 | 264 | 221 | 215 | 1 (no. 8) | 5 |
| " | 4 | 195* | 154 | 152 | 1 (no. 12) | 1 |
| Lex. B-1 | Total | 912 | 674 | 661 | 2 | 11 |
| Lex. B-2 | 1 | 106* | 72 | 68 | 0 | 4 |
| " | 2 | 250* | 147 | 142 | 2 (nos. 26, 27) | 3 |
| " | 3 | 284* | 176 | 171 | 0 | 5 |
| " | 4 | 111* | 82 | 80 | 0 | 2 |
| " | 5 | 113* | 94 | 92 | 0 | 2 |
| Lex. B-2 | Total | 864 | 571 | 553 | 2 | 16 |
| Two F ₁ plants | Grand total | 1,776 | 1,245 | 1,214 | 4 | 27 |

* Indicates seeds from the same capsule.

TABLE VIII
ANALYSIS OF F₁ SEEDLING CULTURES OF "LEXINGTON F"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-------------|-----------------------|--------------|---------|------------------------|---------------------------------|
| 1 | 92* (large, immature) | 68 | 62 | 1 (no. 1) | 5 |
| 2 | 139* (mature) | 34 | 31 | 0 | 3 |
| Total | 231 | 102 | 93 | 1 | 8 |

* Indicates seeds from the same capsule.

TABLE IX
ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON G"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|------------|----------------------------|--------------|---------|------------------------|---------------------------------|
| 1 | 87* (very large, immature) | 25 | 25 | 0 | 0 |
| 2 | 50* (large, immature) | 17 | 17 | 0 | 0 |
| 3 | 285* | 157 | 152 | 0 | 5 |
| 4 | 160 | 140 | 139 | 0 | 1 |
| 5 | 159* | 136 | 133 | 2 (nos. 47, 49) | 1 |
| 6 | 152* | 95 | 93 | 0 | 2 |
| 7 | 187* | 153 | 152 | 0 | 1 |
| 8 | 144* | 86 | 80 | 0 | 6 |
| 9 | 120* | 106 | 99 | 0 | 7 |
| 10 | 197* | 157 | 154 | 1 (no. 54) | 2 |
| 11 | 133* | 98 | 97 | 0 | 1 |
| 12 | 147* | 114 | 110 | 1 (no. 52) | 3 |
| Total | 1,821 | 1,284 | 1,251 | 4 | 29 |

* Indicates seeds from the same capsule.

TABLE X
ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON H"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-------------|---------------|--------------|---------|------------------------|---------------------------------|
| 1 | 301* | 152 | 145 | 2 (nos. 3 and 4) | 5 |
| 2 | 157* | 78 | 76 | 0 | 2 |
| Total | 458 | 230 | 221 | 2 | 7 |

* Indicates seeds from the same capsule.

TABLE XI
ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON I"

| Culture | Seeds planted | Total plants | Typical | Mut. <i>nummularia</i> | Other muts. and suspected muts. |
|-----------|-----------------|--------------|---------|------------------------|---------------------------------|
| 1 | 125* | 57 | 53 | 0 | 4 |
| 2 | 266* | 147 | 145 | 1 (no. 13) | 1 |
| 3 | 244* (immature) | 64 | 61 | 2 (nos. 14 and 15) | 1 |
| Total ... | 635 | 268 | 259 | 3 | 6 |

* Indicates seeds from the same capsule.

TABLE XII

SUMMARY OF TABLES II-XI, SHOWING THE FREQUENCY WITH WHICH
O. pratincola GIVES RISE TO MUT. *nummularia*

| Strain | Generation | Number of seeds | Number of plants | Germination per cent | Number of mut. <i>nummularia</i> | Ratio of mut. <i>nummularia</i> to seeds planted | Ratio of mut. <i>nummularia</i> to total plants |
|-------------|---------------------------------|-----------------|------------------|----------------------|----------------------------------|--|---|
| Lex. A .. | F ₁ | 1083 | 255 | 23.5 | 4 | 1:271 | 1:64 |
| " .. | F ₂ | 719 | 436 | 60.6 | 3 | 1:240 | 1:145 |
| Lex. A .. | F ₁ & F ₂ | 1,802 | 691 | 38.4 | 7 | 1:257 | 1:99 |
| Lex. B .. | F ₁ | 920 | 508 | 55.2 | 0 | | |
| " .. | F ₂ | 1776 | 1245 | 70.1 | 4 | 1:444 | 1:311 |
| Lex. B .. | F ₁ & F ₂ | 2,696 | 1,753 | 65.0 | 4 | 1:674 | 1:438 |
| Lex. C .. | F ₁ | 4221 | 2923 | 69.3 | 11 | 1:384 | 1:266 |
| " .. | F ₂ | 7902 | 5784 | 73.2 | 18 | 1:439 | 1:321 |
| Lex. C .. | F ₁ & F ₂ | 12,123 | 8,707 | 71.8 | 29 | 1:418 | 1:300 |
| Lex. F .. | F ₁ | 231 | 102 | 44.2 | 1 | 1:231 | 1:102 |
| Lex. G .. | F ₁ | 1,821 | 1,284 | 70.5 | 4 | 1:455 | 1:321 |
| Lex. H .. | F ₁ | 458 | 230 | 50.2 | 2 | 1:226 | 1:115 |
| Lex. I. . . | F ₁ | 635 | 268 | 42.2 | 3 | 1:212 | 1:89 |
| All. | F ₁ | 9,369 | 5,570 | 59.5 | 25 | 1:375 | 1:223 |
| " | F ₂ | 10,397 | 7,465 | 71.8 | 25 | 1:416 | 1:299 |
| All. | F ₁ & F ₂ | 19,766 | 13,035 | 66.0 | 50 | 1:395 | 1:261 |

In all, there were 19,766 seeds sown of the 7 strains which gave rise to mut. *nummularia*. They gave 13,035 seedlings, of which 5,570 belonged to F₁ and 7,465 to F₂ progenies. The average germination of the F₁ seeds was 59.5 per cent, or 58 per cent if the 1000 seeds of Lexington C sown in the winter of 1912-1913 are not figured in. Most of the F₁ seeds were over a year old when they were planted. The germination of the F₂ seeds, which were sown soon after they were harvested, was 71.8 per cent. Inspection of table XII shows the remarkable fact that the ratio of mut. *nummularia* to seeds planted was nearly identical for the F₁ and F₂ progenies, 1:375 in the one case, 1:416 in the other, but that the

ratio of *nummularia* mutations to plants showed a variation roughly commensurate with the difference in germinability between the F_1 and F_2 seeds. In other words, the mortality among the year-old F_1 seeds appears to have been largely confined to seeds of typical *O. pratincola*. The ratio of *nummularia* mutations to seeds planted is seen from table XII to be reasonably constant for all 7 strains in both the F_1 and F_2 generations. The ratio of mutations to total plants, however, varies between wide limits, and in every case a low percentage of germination is associated with a high frequency of mutation. The F_1 progeny of Lexington A, for example, included 4 individuals of mut. *nummularia* among 255 plants, a ratio of 1:64. These 255 plants, however, were obtained by sowing 1,083 seeds, of which only a small proportion (23.5 per cent) germinated. There seems no escape from the conclusion that the percentage of germinable seeds of mut. *nummularia* had increased by virtue of the greater mortality among the seeds of typical *O. pratincola*.

The evolutionary significance of differential mortality is too obvious to require any lengthy discussion. Mut. *nummularia* has a distinctly greater survival value than its parent when subjected to conditions which delay germination. It has already been shown that mut. *nummularia* has an enormously greater chance to survive than typical *O. pratincola* when subjected to certain unfavorable soil conditions. These facts should be carefully weighed by critics of the mutation theory who persist in assuming, as a matter of course, that mutations would have no chance to survive in competition with the more numerous typical plants. DE VRIES²⁰ has already shown that the percentage of mutation in a culture of *O. Lamarckiana* from seeds 5 years old was 40 per cent instead of the usual 6 per cent. In his comment on this remarkable result he states that in general the seeds of the mutation remain germinable longer than those of typical *O. Lamarckiana*, and suggests that it might be possible to make use of differential mortality to increase the proportion of mutations in seeds, and thereby to facilitate the discovery of the mutations. The writer unconsciously put this suggestion to a test at the time mutations

²⁰ DE VRIES, H., Die Mutationstheorie 1:186. 1901.

were first found in *O. pratincola*. HUNGER²¹ has recently recorded observations on selective mortality in the seeds of *O. Lamarckiana* which can only be interpreted as showing that the mutations of this species have decidedly a greater survival value than the parent form.

It is often remarked that the *Onagras* are not most usually found in undisturbed habitats with other native plants, but rather as weeds in fields and waste places, among the aliens of our flora. Wherever the soil is disturbed, as by plowing, road-making, excavating, they are frequently found in large numbers. They often dominate the flora on made land and on new railroad embankments, but are for the most part replaced by other weeds when the soil ceases to be disturbed at intervals. A fallow field which contains many *Onagras* for a season or two after cultivation is discontinued will thereafter contain fewer each year. If again plowed, it will apparently be restocked by the germination of seeds which have lain dormant, perhaps for years. Selective mortality among dormant seeds might result in such a field being restocked with plants among which mutations would be unexpectedly numerous.

The most interesting fact shown by table XII is that the frequency of mut. *nummularia* cannot correspond with any Mendelian ratio except that of a tetrahybrid splitting in the ratio 255:1. In the case of a number of progenies, to be sure, the ratio of mutations to plants more nearly approximates the trihybrid ratio 63:1, but it has already been shown that in each such instance the high mutation ratio is associated with a low percentage of germination. When the ratio of mutations to seeds is dealt with, there is no case of an approximation to the 63:1 ratio. The data of table XII, recalculated, are stated in table XIII in such form as to show that no single progeny was large enough to prove that the 255:1 ratio might not be the true one. On the contrary, the data afford no reason to believe that the mutation ratio is 255:1. It may be because of the smallness of the cultures that no single progeny shows a significant deviation from this ratio.

²¹ HUNGER, F. W. T., Recherches expérimentales sur la mutation chez *Oenothera Lamarckiana*, exécutées sous les tropiques. Ann. Jard. Buitenzorg 27:92-113. 1913.

Turning again to table XII, it is seen that the progenies might possibly be assembled in two groups, those with a mutation ratio of approximately 400:1 (group I of table XIII), and those with a ratio of about 250:1 (group II of table XIII). Testing separately the ratios from these groups (see table XIII), we find that the number of individuals in group II is too small to establish a significant deviation from the ratio for group I. (The difference is 0.18 ± 0.13 per cent). It is therefore impossible to demonstrate either that the mutation ratio is or that it is not the same for all the progenies.

TABLE XIII

TEST OF THE FITNESS OF THE MUTATION RATIOS TO THE NEAREST MENDELIAN RATIO (235:1)

| Culture | Group | Class 0 per cent not mut. <i>nummularia</i> | Class 1 per cent mut. <i>nummularia</i> | Number of seeds planted (n.) | Standard deviation ($\sigma = \sqrt{\%ps. \%ps.}$) | Mean error in per cent ($m = \frac{\sigma}{\sqrt{n}}$) | Expectation for each value of n if ratio is 255:1 | Difference between observation and expectation |
|-----------------------|-------|--|--|---------------------------------|---|---|---|--|
| Lex. A-F ₁ | II | 99.63 | 0.37 | 1,083 | 6.07 | 0.18 | 0.39 ± 0.19 | 0.02 ± 0.26 |
| Lex. A-F ₂ | II | 99.58 | 0.42 | 719 | 6.47 | 0.24 | 0.39 ± 0.24 | 0.03 ± 0.34 |
| Lex. B-F ₂ | I | 99.77 | 0.23 | 1,776 | 4.79 | 0.11 | 0.39 ± 0.15 | 0.16 ± 0.29 |
| Lex. C-F ₁ | I | 99.74 | 0.26 | 4,221 | 5.10 | 0.08 | 0.39 ± 0.10 | 0.13 ± 0.13 |
| Lex. C-F ₂ | I | 99.77 | 0.23 | 7,902 | 4.79 | 0.05 | 0.39 ± 0.07 | 0.16 ± 0.09 |
| Lex. F-F ₁ | II | 99.57 | 0.43 | 231 | 5.42 | 0.42 | 0.39 ± 0.41 | 0.04 ± 0.59 |
| Lex. G-F ₁ | I | 99.78 | 0.22 | 1,821 | 4.68 | 0.11 | 0.39 ± 0.15 | 0.17 ± 0.29 |
| Lex. H-F ₁ | II | 99.56 | 0.44 | 458 | 6.59 | 0.31 | 0.39 ± 0.29 | 0.05 ± 0.43 |
| Lex. I-F ₁ | II | 99.53 | 0.47 | 635 | 6.86 | 0.36 | 0.39 ± 0.25 | 0.08 ± 0.44 |
| Group I | | 99.76 | 0.24 | 15,720 | 4.89 | 0.04 | 0.39 ± 0.05 | 0.15 ± 0.06 |
| Group II | | 99.58 | 0.42 | 3,126 | 6.47 | 0.12 | 0.39 ± 0.11 | 0.03 ± 0.16 |
| Groups I & II | | 99.75 | 0.25 | 18,846 | 5.00 | 0.04 | 0.39 ± 0.05 | 0.14 ± 0.06 |
| Total | | 99.75 | 0.25 | 19,766 | 5.00 | 0.04 | 0.39 ± 0.04 | 0.14 ± 0.06 |

If we assume that it is justifiable to treat all of the progenies as one group, the numbers are then large enough to indicate, not however without considerable doubt, that the frequency of occurrence of mut. *nummularia* is not in accord with the tetrahybrid ratio 255:1, but with some ratio lying between 330:1 and 450:1. Of course we cannot assume that there is no mortality at all among the seeds which produce mut. *nummularia*. If in the 30 per cent of seeds *O. pratincola* which never germinate even when fresh the mortality among mutations and non-mutations were the same, then the mutation ratio would not significantly deviate from 255:1. It

is obvious that a Mendelian explanation of the occurrence and frequency of mut. *nummularia* involves the assumption that each parent plant which gave rise to it was heterozygous with regard to at least four factors. Otherwise no segregate would occur with so low a frequency as 1:255. The following objections to a Mendelian explanation may be enumerated:

1. *O. pratincola* is probably almost invariably self-pollinated in a state of nature, for the anthers burst in contact with the receptive stigma the day before the flowers open. In a very few generations heterozygosis would be eliminated from a strain which had accidentally become crossed. Hybridization involving four factors, followed by several generations of self-pollination, would result in an F_2 with 6.25 per cent of homozygotes, an F_3 with 31.64 per cent, F_4 with 58.62 per cent, F_5 with 93.75 per cent, F_6 with 96.87 per cent, F_7 with 98.44 per cent, F_8 with 99.22 per cent, F_9 with 99.61 per cent, F_{10} with 99.80 per cent, etc. It would be utterly absurd to suggest that out of 8 wild mother plants growing far apart, selected at random, 7 were tetrahybrids.

2. An F_1 tetrahybrid would invariably show segregation in a 255:1 ratio. Out of its F_2 progeny, however, only one plant in 16 would be a tetrahybrid, and therefore only one F_2 plant in 16 could exhibit 255:1 segregation in the F_3 . The other F_2 heterozygotes would be hybrids of a lower order. Some would segregate in the ratio 63:1, some in the ratio 15:1, and some in the ratio 3:1. It has already been pointed out (see tables III, V, and VII, summarized in tables XII and XIII) that every F_1 (that is, F_1 with regard to the wild mother plants from Lexington) plant of which seeds were planted either yielded a progeny containing no *nummularia* mutations, in which case the number was not large enough to be sure of getting this mutation, or else the only Mendelian ratio indicated as possible was 255:1. In all, 11 F_2 progenies were grown, of which only 2 failed to give the mutation. The only uncomplicated Mendelian explanation requires that in picking 11 mother plants at random from among 142 F_1 plants, 9 were selected from that one-sixteenth of the culture which was still heterozygous for four characters. It may be pointed out that among 142 plants, just 9 tetrahybrids might reasonably be expected. The chances

are infinitesimal that all 9 would be included among 11 plants chosen at random.

3. A tetrahybrid might give as many as 16 phaenotypes in the F_2 . All of these would have a greater frequency than 1:255 except the pure recessive. We have seen that mut. *nummularia* cannot have a greater frequency than 1:255, and have also seen that it is not a pure recessive, for in the next generation after it originates it gives rise to several distinct types.

4. In the case of one F_2 progeny (Lexington C-91, see table III) from a single mother plant, 1,539 seeds from 6 capsules gave 3 specimens of mut. *nummularia*, whereas 337 seeds from one capsule gave 5. Such a result shows a frequency varying from 1:60 to 1:513 on capsules from the same spike. From a Mendelian standpoint it is practically impossible to explain such a result.

The mutation phenomenon in *O. pratincola* cannot be explained away by any reasonably plausible stretching of Mendelian theory. On the contrary, it seems obvious that mutation is quite a different process from hybrid segregation, although both processes may occur simultaneously.

Mut. *nummularia* is the only one of the mutations of *O. pratincola* the frequency of which has been determined. None of the others has been observed throughout the complete cycle from seed to seed and carried into a second generation. In tables II–XI all of the variants except mut. *nummularia* are thrown together as “other mutations or suspected mutations.” In explanation of this mixed category, it is necessary to state that all unusually small or unusually large plants, regardless of whether or not they appeared otherwise different from the mass of the culture, were counted as “suspected mutations,” in order to be sure that no such variation as a mut. *nanella* would be passed over. To judge from past experience, most of the “suspected mutations” will develop as quite normal plants. Consequently the mutability of *O. pratincola* is probably not as great as might be assumed from the tables.

Mut. *Mummularia* a discontinuous variation

Critics of DE VRIES' work on mutation in *Oenothera Lamarckiana* have not infrequently expressed skepticism as to whether or not the

mutations were actually unconnected with the parent form by intermediates, which might have been overlooked in classifying the young seedlings. An endeavor has been made to forestall the same criticism of the writer's work on *O. pratincola* by the publication

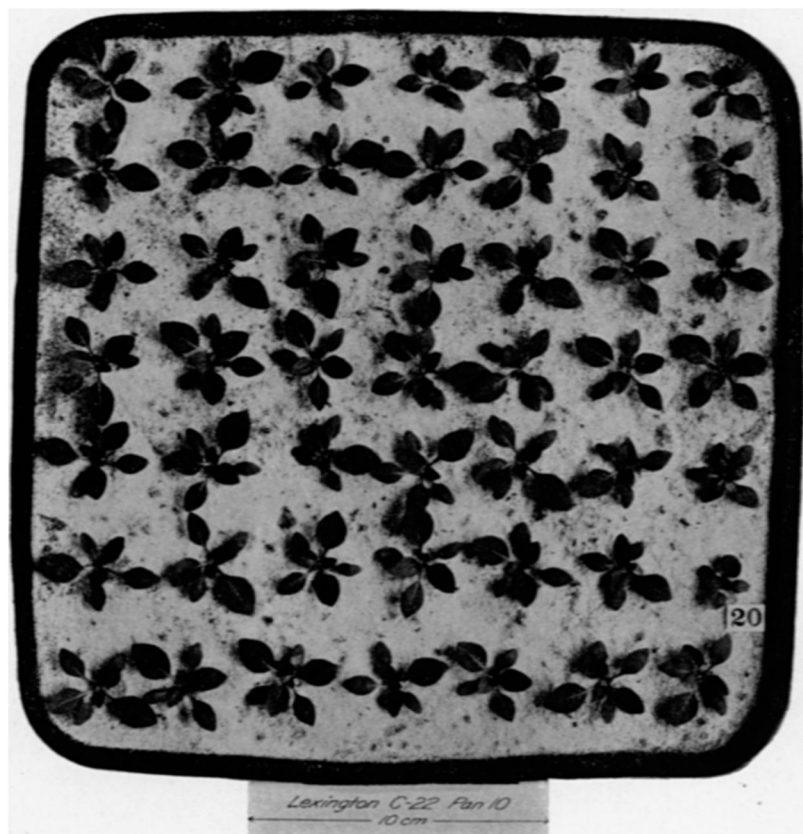


FIG. 12.—F₂ progeny of Lexington C, *Oenothera pratincola* (pan 10 of the progeny of C-22); the only mutation shown is C-22-20, mut. *nummularia*; the other plants are typical *O. pratincola*.

of a series of photographs showing some of the pans in which the mutations occurred. Each reader can judge for himself as to the discontinuity of the mutations from the rest of the plants. It is believed that no one has heretofore published so extensive a series of photographs representing random samples of cultures from which

none of the seedlings had been discarded. It must be remembered that each pan is a fair sample of a whole culture, for the seedlings were pricked off when very small and were taken from the seed pan as they came, with no attempt at sorting.



FIG. 13.—F₂ progeny of Lexington C, *Oenothera pratincola* (pan 17 of the progeny of C-52); one example of mut. *nummularia*, C-52-28, is shown; the remaining plants are typical.

Figs. 5, 6, 12, 13, 14, 15, and 17 show 7 of the 50 occurrences of mut. *nummularia* in cultures aggregating 13,035 plants. Three more of the original plants of this mutation are shown in figs. 3, 4, and 16. The figures showing entire pans should give a fairly clear idea of what the writer interpreted as fluctuating variation. It is believed that very few if any mutations escaped detection in the

cultures of 1914. The two mutations of Lexington C (nos. 28 and 57) which passed muster as typical plants when the seedlings of 1913 were examined would probably not have been missed in the

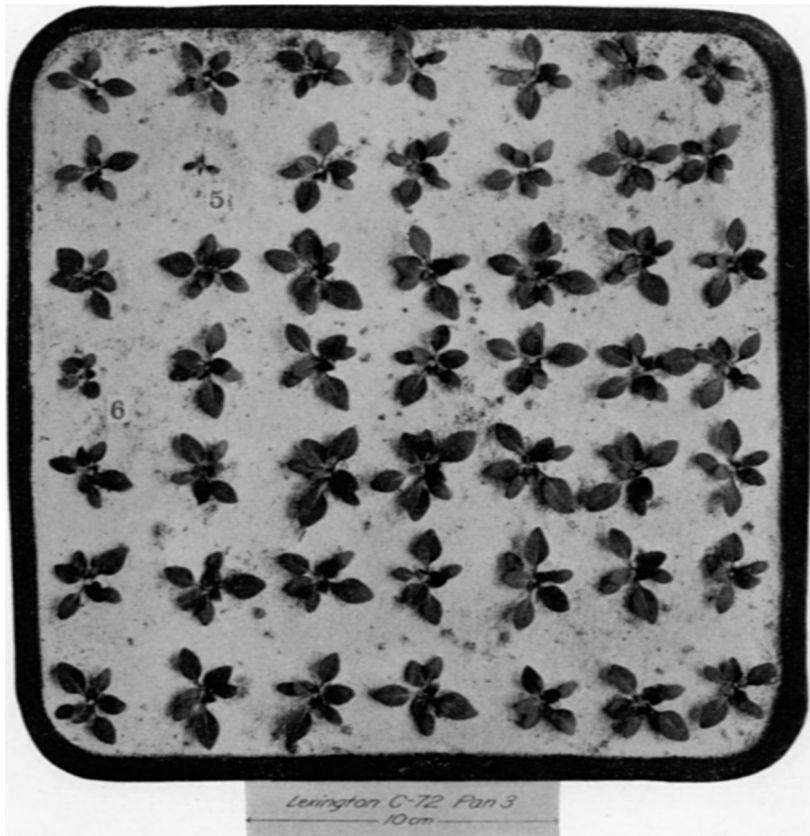


FIG. 14.—F₂ progeny of Lexington C, *Oenothera pratincola* (pan 3 of the progeny of C-72); two mutations are shown, mut. *subulata*, C-72-5, and mut. *nummularia*, C-72-6; the other plants are typical.

more searching scrutiny which the seedlings of 1914 underwent.²² Although some of the mutations cannot be distinguished in the young seedling stage with ease, it is believed that the likelihood

²² Mut. *nilida*, represented by Lexington C-57 in the cultures of 1913, occurred several times in 1914 and was detected in the young seedling stage in every case Nov. (1914).

of mistaking mut. *nummularia* for the parent type or for one of the other mutations is negligible. The orbicular seedling leaves are too striking a characteristic to be overlooked.

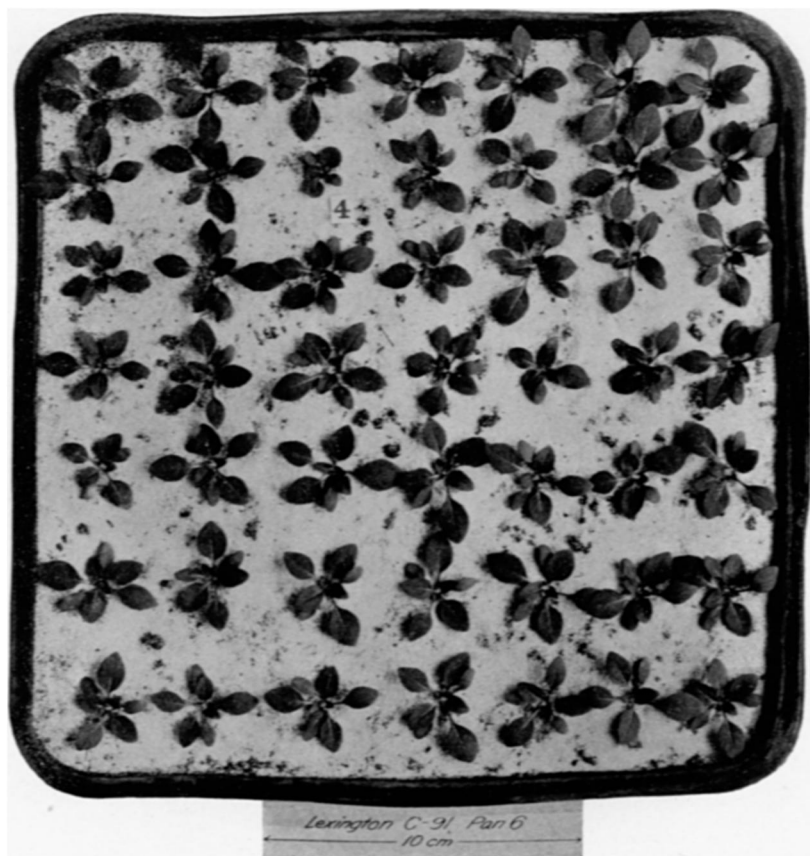


FIG. 15.—F₂ progeny of Lexington C, *Oenothera pratincola* (pan 6 of the progeny of C-91); one plant of mut. *nummularia* is shown, C-91-4; the other plants are typical.

The unlikeness of mut. *nummularia* and *O. pratincola* × *O. numismatica*

Before mut. *nummularia* had been found in F₂ progenies from guarded seed, it seemed possible that it might be an F₁ hybrid of *O. pratincola* with some other wild species, of which a few pollen

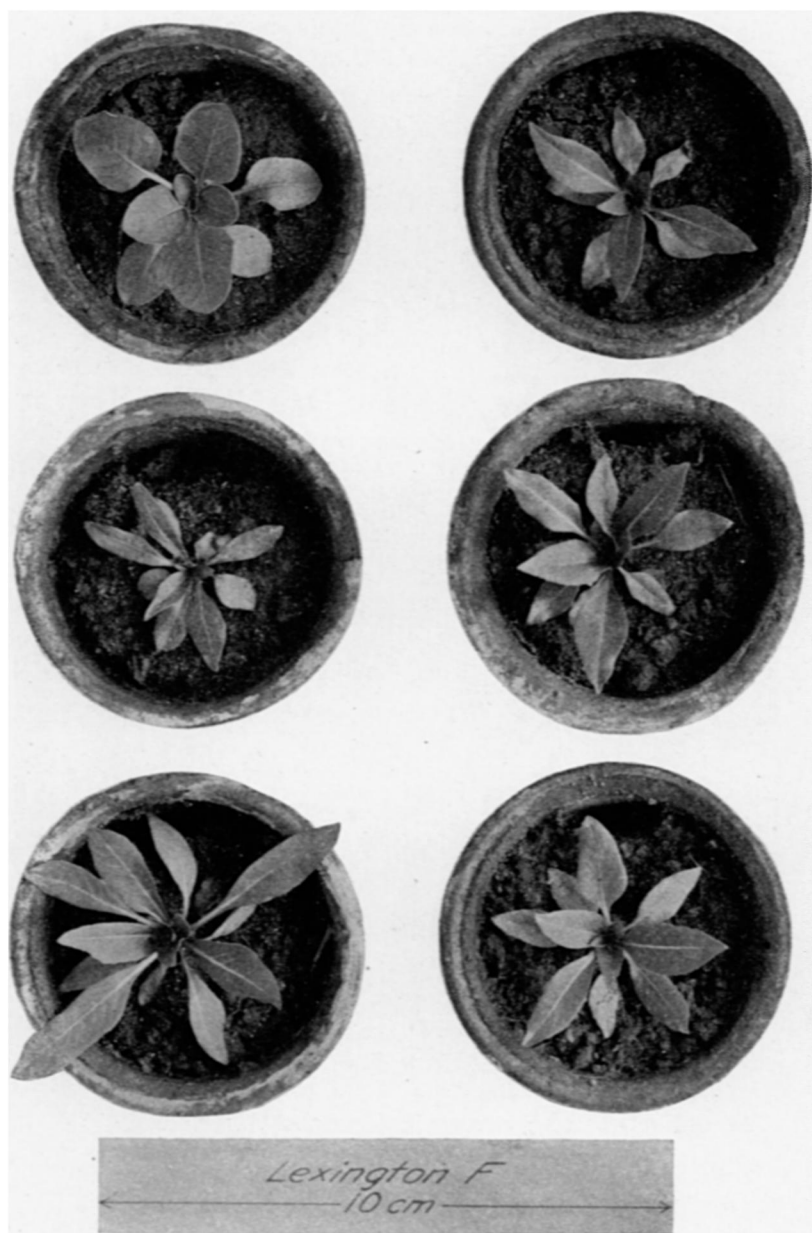


FIG. 16.—Mutations from the F_1 progeny of Lexington F, *Oenothera pratincola*; the plants are F-1, mut. *nummularia*; F-2, -4, -5, -6, mut. *rubricentra*; F-3, mut. *tortuosa* (?), taking the plants in order from the upper left-hand corner.

grains had accidentally reached the stigmas of the mother plants. This hypothesis was tested by crossing *O. pratincola* with *O. numismatica*. As already stated, these two species were the only *Onagras* which the writer found at Lexington. The latter, furthermore, is



FIG. 17.—F₁ progeny of Lexington G, pan 27, *Oenothera pratincola*; four mutations are shown, G-50 and G-53, mut. *rubricentra*; G-51, mut. *nilida*; G-52, mut. *nummularia*; the other plants are typical *O. pratincola*.

suspiciously similar in several characters to mut. *nummularia*, as may be seen by comparing the characters already recorded. The cross *O. pratincola* ♀ × *O. numismatica* ♂ was conspicuously fertile; 326 seeds from one capsule gave a culture of 222 plants, consisting

of twin hybrids and one mutation. The solitary exception was broader leaved than the rest of the culture and is interpreted by the writer as the product of a cross between a mutated ♀ gamete, which if pollinated by *O. pratincola* would have yielded mut. *nummularia*, and a normal ♂ gamete of *O. numismatica*. This hypothesis will be tested later by appropriate crosses. It is clear that a cross between *O. pratincola* and *O. numismatica* does not yield mut. *nummularia*, or anything resembling it, with greater frequency than does unhybridized *O. pratincola*. Perhaps *O. numismatica* is itself a mutation from *O. pratincola*, or a form which has segregated from the cross mut. *nummularia* × *O. pratincola*. One would expect the latter cross to occur rather often if, as seems to be the case, mut. *nummularia* itself is partially self-sterile. It seems not unlikely that in nature self-sterile or nearly self-sterile mutations may be perpetuated by effective cross-pollination, either as stable hybrids or as homozygous forms resulting from subsequent segregation. It is an interesting fact that although *O. pratincola* has a very high proportion of good pollen grains (90 per cent or more), mut. *nummularia* rarely has pollen which is 50 per cent perfect, and some anthers produce no good pollen at all.

Conclusions

1. *Oenothera pratincola*, a recently described small-flowered self-pollinating species from Kentucky, is in a mutating condition comparable with that of *O. Lamarckiana*.

2. The most striking of the mutations, *O. pratincola* mut. *nummularia*, occurred in strains derived from 7 wild mother plants out of 8 selected at random.

3. In two of these strains the mutation was found in both the F_1 and F_2 generations from the parent plant. In a third strain the mutation was found only in the F_2 generation, but a sufficient number of F_1 plants had not been grown to insure its detection in that generation.

4. Mut. *nummularia* appears to occur with a frequency of about one individual to each 300-400 seeds planted. The several progenies showed no significant variation in the mutation ratio.

5. The mutation ratio cannot be explained on Mendelian grounds.

6. Mut. *nummularia* is better adapted than the parent type to withstand influences unfavorable to germination. In every case where a progeny contained an unexpectedly large number of mutations, the germination was correspondingly poor.

7. Selective germination and differential mortality among dormant seeds may be important factors in natural selection.

8. Mutation is a distinct process from Mendelian segregation, and the phenomena exhibited by *Oenothera Lamarckiana*, *O. biennis*, and *O. pratincola* cannot be attributed to heterozygosis.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.